Deinococcus ficus sp. nov., isolated from the rhizosphere of *Ficus religiosa* L.

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A pale-pink strain (CC-FR2-10^T) from the rhizosphere of the sacred tree *Ficus religiosa* L. in Taiwan was investigated by using a polyphasic taxonomic approach. The cells were Gram-positive, rod-shaped and non-spore-forming. Phylogenetic analyses using the 16S rRNA gene sequence of the isolate indicated that the organism belongs to the genus *Deinococcus*, the highest sequence similarities being found with *Deinococcus grandis* (96·1 %), *Deinococcus radiodurans* (94·3 %), *Deinococcus radiopugnans* (93·2 %), *Deinococcus indicus* (93·0 %), *Deinococcus proteolyticus* (92·5 %), *Deinococcus murrayi* (92·4 %) and *Deinococcus geothermalis* (90·7 %). The DNA–DNA relatedness with respect to *D. grandis* DSM 3963^T was 17·9 %. Chemotaxonomic data revealed that strain CC-FR2-10^T contains only menaquinone MK-8 as the respiratory quinone, unknown phosphoglycolipids as the predominant polar lipids and 16 : 1ω7*c*, 17 : 1ω8*c* and 17 : 1ω9*c* iso as the predominant fatty acids. The biochemical and chemotaxonomic properties demonstrate that strain CC-FR2-10^T represents a novel species, for which the name *Deinococcus ficus* sp. nov. is proposed. The type strain is CC-FR2-10^T (=CCUG 53391^T=CIP 108832^T).

At the time of writing, the genus *Deinococcus* comprises nine species with validly published names, *Deinococcus radiodurans* (the type species), *D. erythromyxa* (transferred to the genus *Kocuria* by Rainey *et al.*, 1997), *D. geothermalis*, *D. grandis*, *D. indicus*, *D. murrayi*, *D. proteolyticus*, *D. radiophilus* and *D. radiopugnans*. These species and the taxonomy of the genus have been extensively studied (Suresh *et al.*, 2004; Ferreira *et al.*, 1997; Rainey *et al.*, 1997; Brooks & Murray, 1981). In addition, the names of nine further species have recently been effectively published: *Deinococcus hohokamensis*, *D. navajonensis*, *D. hopiensis*, *D. apachensis*, *D. maricopensis*, *D. pimensis*, *D. yavapaiensis*, *D. papagonensis* and *D. sonorensis* (Rainey *et al.*, 2005) (these names have subsequently been validly published). Furthermore, three species have been described whose names have not yet been validly published; '*Deinococcus frigens*', '*D. saxicola*' and '*D. marmoris*' (Hirsch *et al.*, 2004). 16S rRNA gene sequence data for an additional species, *Deinococcus deserti*, are already available (this name has since been validly published; de Groot *et al.*, 2005). Several novel *Deinococcus* strains have been isolated from soils, desert soil, foods, faeces and dust, and have been characterized in detail; there are additional data on their extreme resistance to UV light, gamma radiation and desiccation, which is a distinctive characteristic of this genus, being present in almost every species. In members of the genus *Deinococcus*, ionizing radiation and desiccation induce similar types of DNA damage, and it is has been proposed that resistance to unnaturally large amounts of ionizing radiation is a consequence of the ability to repair desiccation-induced DNA damage (Mattimore & Battista, 1996). Recently, the extensive diversity of this genus was recorded, and nine novel and extremely ionizing radiation-resistant bacteria isolated from desert soil have been described (Rainey *et al.*, 2005). Although the aforementioned properties of members of this genus are well characterized, the functional roles of *Deinococcus* in rhizosphere soil or in plant growth promotion remain largely unexplored.

During screening for effective plant-growth-promoting rhizobacteria from the rhizosphere of the tree *Ficus religiosa* L., a pale-pink-pigmented bacterium was isolated on nutrient

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CC-FR2-10^T is AY941086.

A supplementary table showing the fatty acid profiles of strain CC-FR2-10^T and representative *Deinococcus* species and a phylogenetic tree constructed using maximum parsimony are available as supplementary material in IJSEM Online.

agar. This strain (CC-FR2-10^T) was maintained and subcultured on nutrient agar at 30 °C for 48 h. The 16S rRNA gene sequence, fatty acid methyl ester composition of whole-cell hydrolysates, respiratory quinones and polar lipids were then determined. Additional phenotypic analyses were performed: biochemical tests, carbon-source utilization (Biolog GP2), API ZYM enzyme profiles (bioMérieux), API 20E (bioMérieux), UV radiation resistance and DNA–DNA relatedness to *D. grandis* (the most closely related species in terms of 16S rRNA gene sequence similarity).

Cultural and morphological characteristics were observed on nutrient agar and Degryse agar (Degryse *et al.*, 1978). Flexirubin-like pigments were observed by flooding the plates with 20 % (w/v) KOH (Fautz & Reichenbach, 1980). The Gram reaction was tested by using the modified method of Cowan (1974). Motility was tested under a microscope, using cells grown for 3 days in motility-test, semi-solid medium at 30 °C. Fluorescence was tested after 48 h by means of plating on King's B agar. The pH range for growth was tested in Degryse medium as described by Ferreira *et al.* (1997).

Strain CC-FR2-10^T was Gram-positive and formed visible (about 2 mm), pale-pink colonies after 48 h at 30 °C. No growth was observed at temperatures above 42 °C. The colonies were translucent and shiny with entire edges. A pale-pink pigment was produced on nutrient agar: this pigment was non-diffusible, non-fluorescent and did not change upon the addition of 20 % KOH. Oxidase activity was tested for by using oxidase reagent (bioMérieux) according to the instructions of the manufacturer. The cells of strain CC-FR2-10^T were oxidase-positive, non-motile, non-spore-forming rods. Strain CC-FR2-10^T was able to grow well on nutrient agar and Degryse agar. Optimum growth was observed at alkaline pH; the strain could tolerate, and grow at, pH 10.

Physiological characterization and additional biochemical tests were performed to assess the carbon-source utilization pattern, using Biolog GP2 plates, and the hydrolysis of 19 substrates was investigated using the API ZYM system and API 20E according to the methods outlined by the manufacturer (bioMérieux).

UV irradiation was carried out according to the methods outlined by Hirsch *et al.* (2004), under a 254 nm UV lamp; *Escherichia coli* served as a control.

The fatty acid pattern of strain CC-FR2-10^T was determined using the method described by Kämpfer & Kroppenstedt (1996). The pattern is compared with those of some representative *Deinococcus* species in Supplementary Table S1 available in IJSEM Online. Strain CC-FR2-10^T had a fatty acid profile typical of members of the genus *Deinococcus*.

The respiratory quinones were extracted and analysed by HPLC as described by Tindall (1990) and Altenburger *et al.* (1996). The quinone system of strain CC-FR2-10^T consisted solely of menaquinone MK-8. This corresponds with other *Deinococcus* species, all of which contain MK-8 as the major quinone (Embley *et al.*, 1987; Ferreira *et al.*, 1997; Oyaizu *et al.*, 1987; Suresh *et al.*, 2004).

Polar lipids were extracted and analysed by two-dimensional TLC according to Tindall (1990). Like other *Deinococcus* species (Embley *et al.*, 1987; Suresh *et al.*, 2004; Ferreira *et al.*, 1997), strain CC-FR2-10^T displayed a complex polar lipid profile consisting of various unknown glycolipids, phosphoglycolipids and phospholipids and an unknown aminophospholipid; an unknown phosphoglycolipid was the predominant component (Fig. 1).

The 16S rRNA gene was amplified by using a PCR with bacterial universal primers 1F and 9R (Kämpfer *et al.*, 2003; Shen *et al.*, 2005). PCR products were purified from agarose gel using the QIAquick Gel extraction kit (Qiagen). The sequencing primers used were 3F (5′-CCTACGGGAGG-CAGCAG-3′, corresponding to positions 341–357 of *E. coli*), 4R (5′-TTACCGCGGCTGCTGGCAC-3′; positions 533–515) and 5F (5′-AAACTCAAATGAATTGACGGGG-3′; positions 907–928) (Brosius *et al.*, 1978; Edwards *et al.*, 1989). Sequence analysis was performed using an ABI PRISM 310 DNA sequencer (Applied Biosystems), sequence assembly was performed using the Wisconsin Package, version 9.1 (GCG) with a Fragment Assembly System program supplied by the National Health Research Institute of Taiwan. The phylogenetic tree was constructed from the distance matrices by using the neighbour-joining method.

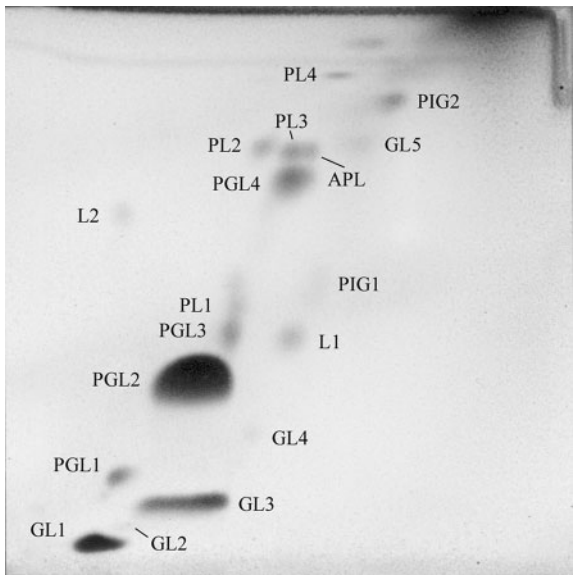

Fig. 1. Polar lipid profile of strain CC-FR2-10^T. L1 and L2, Unidentified polar lipids; GL1–GL5, unidentified glycolipids; PGL1–PGL4, unidentified phosphoglycolipids; PL1–PL4, unidentified phospholipids; APL, unidentified aminophospholipid; PIG1 and PIG2, brick-red pigments. PIG2 stained with α-naphthol, indicating that it contained sugar structures.

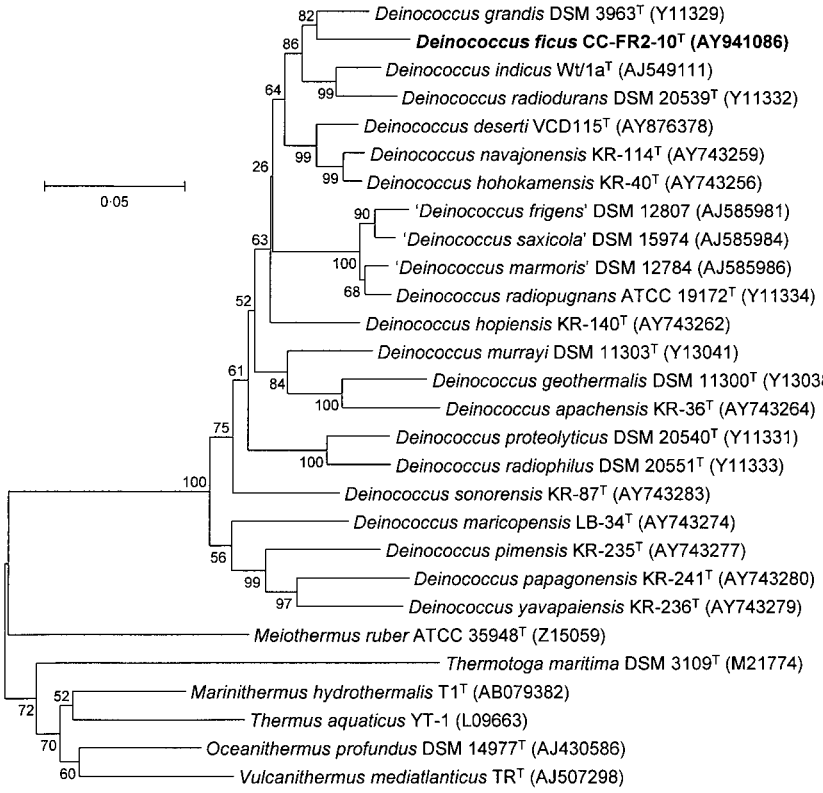

Fig. 2. Phylogenetic tree, based on 16S rRNA gene sequences available from the EMBL database (accession numbers are given in parentheses), constructed after multiple alignment of the data by CLUSTAL X (Thompson *et al.*, 1997). Distances (distance options according to the Kimura-2 model) and clustering with the neighbour-joining method were obtained by using the software package MEGA, version 2.1 (Kumar *et al.*, 2001). Bootstrap values based on 1000 replications are listed as percentages at branching points. Bar, 0·05 K_{nuc} value. A maximum-parsimony tree is available as Supplementary Fig. S1 in IJSEM Online.

Trees were constructed by using neighbour joining (Fig. 2) and maximum parsimony (see Supplementary Fig. S1 available in IJSEM Online). An almost-complete (1453 nt) 16S rRNA gene sequence of CC-FR2-10^T (AY941086) was aligned with sequences deposited in GenBank, using CLUSTAL X (Thompson *et al.*, 1997). This showed that strain CC-FR2-10^T was phylogenetically most closely related to species of the genus *Deinococcus*. According to the gene sequence similarity calculations, the most closely related strain was *D. grandis* DSM 3963^T (96·1 %), followed by *D. radiodurans* DSM 20539^T (94·3 %), *D. radiopugnans* ATCC 19172^T (93·2 %), *D. indicus* Wt-1a^T (93·0 %), *D. proteolyticus* DSM 20540^T (92·5 %), *D. murrayi* DSM 11303^T (92·4 %) and *D. geothermalis* DSM 11300^T (90·7 %). DNA–DNA hybridization experiments were performed with strain CC-FR2-10^T and the type strain of the phylogenetically most closely related *Deinococcus* species, *D. grandis* DSM 3963^T. The method used was that described by Ziemke *et al.* (1998), except that, for nick translation, 2 μg DNA was labelled with incubation at 15 °C for 3 h. Strain CC-FR2-10^T showed relatively low levels of DNA–DNA hybridization with *D. grandis* DSM 3963^T (17·9 %; reciprocal analysis, 14·1 %), which clearly indicated that CC-FR2-10^T represents a distinct species.

Strain CC-FR2-10^T utilized several carbon sources and was able to hydrolyse 12 out of 19 compounds in the API ZYM system. The results of biochemical/physiological tests are given in Table 1 and in the species description. MK-8 was the predominant respiratory quinone of CC-FR2-10^T, as for other *Deinococcus* species, and an unknown phosphoglycolipid was the predominant polar lipid. Strain CC-FR2-10^T was resistant to UV irradiation (254 nm, 8–10 cm for

Table 1. Comparison of the phenotypic characteristics of strain CC-FR2-10^T and *D. grandis* DSM 3963^T

Carbon source utilization was determined with the Biolog GN2 system. Both organisms were short rods, were able to grow at 40 °C, showed nitrate reduction and were positive for oxidase and hydrolysis of aesculin and gelatin. Both strains showed positive results for the utilization of glucose, sucrose (weak in the case of CC-FR2-10^T), fructose and maltose. Both strains produced negative results for the utilization of cellobiose and for arginine dihydrolase, urease, indole and H_2S.

Characteristic	*D. ficus* CC-FR2-10^T	*D. grandis* DSM 3963^T
Pigmentation	Pale pink	Pink/red
Utilization as carbon source:		
L-Arabinose	+	−
Lactose	+	−
D-Trehalose	+	−
D-Xylose	+	−
D-Mannose	+	−
D-Melibiose	+	−
N-Acetyl-D-glucosamine	+	−
D-Sorbitol	+	−

10 min), as are many other *Deinococcus* species (Brooks & Murray, 1981).

On the basis of the results of this polyphasic taxonomic analysis and radiation-resistance studies, it is clear that strain CC-FR2-10^T represents a novel species of the genus *Deinococcus*, for which the name *Deinococcus ficus* sp. nov. is proposed.

Description of *Deinococcus ficus* sp. nov.

Deinococcus ficus (fi′cus. L. n. *ficus* a fig tree and the name of a botanical genus; L. gen. n. *ficus* of *Ficus*, referring to the isolation of the type strain from the rhizosphere of *Ficus religiosa* L.).

Cells are Gram-positive, non-motile, non-spore-forming rods. Aerobic, oxidase-positive and show good growth after 48 h on nutrient agar and tryptic soy agar at 37 °C. Colonies on nutrient agar are smooth, pale pinkish, circular, translucent and shiny with entire edges; colonies become mucoid. Pink pigmentation is non-diffusible, non-fluorescent and does not change upon the addition of 20 % KOH. Unable to grow at 5 or 42 °C. Growth occurs at pH 5·5–10. Resistant to UV irradiation (254 nm, 8–10 cm for 10 min). Major cellular fatty acids are 16 : 1ω7*c*, 17 : 1ω8*c*, 17 : 1ω9*c* iso, 16 : 0, 17 : 0 iso and 15 : 1ω6*c*. MK-8 is the predominant lipoquinone. An unknown phosphoglycolipid is the predominant polar lipid. The following compounds are utilized as sole carbon sources (i.e. produce positive results in the Biolog system): dextrin, Tweens 40 and 80, *N*-acetyl-D-glucosamine, *N*-acetyl-β-D-mannosamine (weakly), L-arabinose, D-fructose, L-fucose, D-galactose, D-galacturonic acid, D-gluconic acid, α-D-glucose, α-D-lactose, maltose, maltotriose, D-mannitol, D-mannose, D-melibiose, methyl α-D-galactoside, methyl β-D-galactoside, methyl β-D-glucoside, D-raffinose, L-rhamnose, D-ribose, D-sorbitol, stachyose, sucrose, D-trehalose, D-xylose, acetic acid, β-hydroxybutyric acid, *p*-hydroxyphenylacetic acid, L-lactic acid, D-malic acid, L-malic acid, pyruvic acid methyl ester, succinic acid monomethyl ester, propionic acid, pyruvic acid, succinic acid, L-alanine, alanyl L-glycine, L-asparagine, L-glutamic acid, glycyl L-glutamic acid, L-serine, putrescine (weakly), glycerol, adenosine, 2-deoxyadenosine, inosine, thymidine, uridine, adenosine 5′-monophosphate, thymidine 5′-monophosphate, uridine 5′-monophosphate, D-fructose 6-phosphate, α-D-glucose 1-phosphate, D-glucose 6-phosphate, DL-α-glycerol phosphate. Positive for β-galactosidase, acetoin production, gelatinase, mannitol oxidation and cytochrome oxidase activity, alkaline phosphatase, butyrate esterase, caprylate esterase, leucine arylamidase, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, α-glucosidase, β-glucosidase, α-mannosidase and α-fucosidase.

The type strain, CC-FR2-10^T (=CCUG 53391^T=CIP 108832^T), was isolated from the rhizosphere of *Ficus religiosa* L.

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